

## CLAIMS

What is claimed is:

1. A packaging cell line for producing a viral accessory protein independent HIV-derived retroviral vector particle comprising:
- 5 a) a mammalian cell;
- b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV *gagpol* proteins;
- c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and
- d) a third retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.
2. A packaging cell line of Claim 1 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).
- 15 3. A packaging cell line of Claim 1 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
4. A packaging cell line of Claim 1 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
5. A packaging cell line comprising:
- a) a mammalian cell;

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- b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV *gagpol* proteins; and
- c) a second retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

6. A packaging cell line of Claim 5 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

7. A packaging cell line comprising:

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- a) a mammalian cell;
- b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV *gagpol* proteins; and
- c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

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8. A method of producing a packaging cell line for producing a viral accessory protein independent HIV-derived retroviral vector particle, comprising co-transfecting mammalian host cells with:

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- a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV *gag* and *pol* proteins;
- b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and

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- c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.
9. A method of Claim 8 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).
10. A method of Claim 8 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
11. A method of Claim 8 wherein the DNA sequence of interest is a heterologous therapeutic protein.
- 10 12. A method of producing a viral accessory protein independent HIV-derived retroviral vector particle comprising co-transfecting mammalian host cells with:
- a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV *gagpol* proteins;
- b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
- c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.
- 20 13. A method of Claim 12 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

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14. A method of Claim 12 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
15. A method of Claim 12 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
- 5 16. A packaging cell line for producing a viral accessory protein independent lentivirus-derived retroviral vector particle comprising:
- a) a mammalian cell;
- b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a lentivirus *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the lentivirus gagpol proteins;
- c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and
- d) a third retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.
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17. A packaging cell line of Claim 16 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).
18. A packaging cell line of Claim 16 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.
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19. A packaging cell line of Claim 16 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

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20. A packaging cell line comprising:

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- a) a mammalian cell;
  - b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the lentivirus *gagpol* proteins; and
  - c) a second retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

10 21. A packaging cell line of Claim 20 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

22. A packaging cell line comprising:

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- a) a mammalian cell;
  - b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the lentivirus *gagpol* proteins; and
  - c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

20 23. A method of producing a packaging cell line for producing a viral accessory protein independent lentivirus-derived retroviral vector particle, comprising co-transfecting mammalian host cells with:

- a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus *gag* and *pol* proteins;

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- b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
  - c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

24. A method of Claim 23 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

25. A method of Claim 23 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

10 26. A method of Claim 23 wherein the DNA sequence of interest is a heterologous therapeutic protein.

27. A method of producing a viral accessory protein independent lentivirus-derived retroviral vector particle comprising co-transfecting mammalian host cells with:

- Sub B8 15
- a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus *gagpol* proteins;
  - b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
  - c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

20 28. A method of Claim 27 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

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29. A method of Claim 27 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

30. A method of Claim 27 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

5 31. A viral accessory protein independent HIV-derived retroviral vector particle produced by the method comprising co-transfecting mammalian host cells with:

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- a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV *gagpol* proteins;
  - b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
  - c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

15 32. A method of Claim 31 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

33. A method of Claim 31 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

20 34. A method of Claim 31 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

35. A viral accessory protein independent lentivirus-derived retroviral vector particle produced by the method comprising co-transfecting mammalian host cells with:

- a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus *gagpol* proteins;
- b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
- c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

36. A method of Claim 35 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

37. A method of Claim 35 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

38. A method of Claim 35 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

39. Isolated DNA encoding a codon optimized HIV *gagpol*.

40. Isolated DNA encoding a codon optimized HIV *gag*.

41. Isolated DNA of Claim 40 comprising the nucleotide sequence of SEQ ID NO:4.

42. Isolated DNA encoding a codon optimized HIV *pol*.

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43. Isolated DNA of Claim 42 comprising the nucleotide sequence of SEQ ID NO:10.
44. A method of introducing a DNA sequence of interest into a mammal comprising introducing into said mammal a viral accessory protein independent HIV-derived retroviral vector particle comprising the DNA sequence of interest.
45. The method of Claim 44 wherein the mammal is a human.
46. The method of Claim 44 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.
47. A method of introducing a DNA sequence of interest into a mammal comprising the steps of:
- a) introducing into cells a viral accessory protein independent HIV-derived retroviral vector particle comprising the DNA sequence of interest; and
  - b) returning the cells obtained in step a) to the mammal.
48. The method of Claim 47 wherein the mammal is a human.
49. The method of Claim 47 wherein the DNA sequence of interest is a heterologous therapeutic protein.